(FILE 'HOME' ENTERED AT 19:08:17 ON 30 NOV 2002)

FILE 'AGRICOLA, ALUMINIUM, ANABSTR, APOLLIT, AQUIRE, BABS, BIOCOMMERCE, BIOTECHNO, CABA, CAOLD, CAPLUS, CBNB, CEABA-VTB, CEN, CERAB, CIN, COMPENDEX, CONFSCI, COPPERLIT, CORROSION, ENCOMPLIT, ENCOMPLIT2, FEDRIP, GENBANK, INSPEC, INSPHYS, INVESTEXT, IPA, ...' ENTERED AT 19:08:54 ON 30 NOV 2002

```
L1     420008 S NUCLEIC ACID
L2       6347 S L1 AND KLENOW FRAGMENT
L3       5806 S L2 AND DNA POLYMERASE
L4        378 S L3 AND TERMINAL TRANSFERASE
L5        171 S L4 AND LABELS
L6          2 S L5 AND CROSSLINKING AGENT
L7          3 S MORPHOLINO(W)NUCLEOTIDE
```

ICS: C07H021-04
EXF 514/44; 536/24.3; 536/24.5; 536/24.31; 536/24.32; 536/18.4; 536/124; 536/23.1; 435/6; 435/172.1; 435/172.3; 435/375; 436/94; 436/183
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l6 1-2, std, kwic

L6 ANSWER 1 OF 2 USPATFULL
AN 2001:121238 USPATFULL
TI Mass spectrometric methods for sequencing nucleic acids
IN Kang, Changwon, Taejon, Korea, Republic of
Kwon, Young-Soo, Kwangju, Korea, Republic of
Kim, Young Tae, Seoul, Korea, Republic of
Koster, Hubert, La Jolla, CA, United States
Little, Daniel P., Patton, PA, United States
Little, Maryanne J., Groton, MA, United States now by change of name from Maryanne J. O'Donnell
Xiang, Guobing, San Diego, CA, United States
Lough, David M., Eyemouth, United Kingdom
Cantor, Charles, Boston, MA, United States
PA Sequenom, Inc., San Diego, CA, United States (U.S. corporation)
PI US 6268131 B1 20010731
AI US 1997-990851 19971215 (8)
DT Utility
FS GRANTED
LN.CNT 3013
INCL INCLM: 435/006.000
INCLS: 435/091.200
NCL NCLM: 435/006.000
NCLS: 435/091.200
IC [7]
ICM: C12Q001-68
EXF 435/6; 435/91.1; 435/91.2; 435/91.3; 435/518; 435/528; 436/518; 436/528; 536/23.1
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
SUMM . . . the unknown DNA sequence area and thereby copying the template and synthesizing a complementary strand using DNA polymerases, such as **Klenow fragment** of E. coli **DNA polymerase** I, a **DNA polymerase** from Thermus aquaticus, Taq **DNA polymerase**, or a modified T7 **DNA polymerase**, Sequenase (e.g., Tabor et al., (1987) Proc. Natl. Acad. Sci. U.S.A. 84:4767-4771), in the presence of chain-terminating reagents. Here, the. . . sequencing DNA require the use of polyacrylamide gel electrophoresis (i.e., PAGE) that can result in sequencing artifacts or require detectable **labels**, such as radioisotopes, enzymes, or fluorescent or chemiluminescent moieties.
SUMM . . . a further object herein to provide methods of sequencing nucleic acids in an array format using RNA polymerase in which **nucleic acid** probes are immobilized to supports at high densities to facilitate mass spectrometric detection. It is also an object herein to. . .
SUMM In certain embodiments, a double stranded **nucleic acid** molecule encoding a promoter sequence is isolated from a natural source (e.g., bacteria, viruses, bacteriophages, plants or eukaryotic organisms) or. . . of the coding strand. This single stranded region is designed such that it is complementary to a region of the **nucleic acid** to be sequenced or to a common overlapping sequence (e.g., a restriction endonuclease site). In preferred embodiments, the promoter-containing **nucleic acid** is covalently coupled via the 3'-end of the noncoding strand or 5'-end of the coding strand to a solid support. . .
SUMM The **nucleic acid** to be sequenced containing at least a partially single stranded 3'-end is hybridized to the complementary

sequences of the promoter-containing DNA. The **nucleic acid** to be sequenced may be single stranded or double stranded. The hybridization of the two **nucleic acid** molecules introduces one or more "nicks" in the hybrid at the junction(s) of the adjacent **nucleic acid** molecules. In certain embodiments, nicks in the coding or non-coding strand, preferably the coding strand, are ligated by the addition of an appropriate **nucleic acid** ligase prior to initiating transcription (i.e., DNA or RNA ligase).

SUMM . . . is analyzed by matrix-assisted laser desorption/ionization mass spectrometry (MALDI) and preferably further uses time-of-flight (TOF) analysis. The sequence of the **nucleic acid** is obtained by aligning the observed mass of the chain-terminated RNA transcripts obtained from sequencing reactions containing each of the. . .

SUMM . . . method of sequencing may be used for diagnostic applications to determine the presence of genetic alterations in a known target **nucleic acid**. For example, a region of the target **nucleic acid** is amplified and the **nucleic acid** strand corresponding to the noncoding strand is isolated. The **nucleic acid** probe containing the promoter may be isolated from a natural source or assembled synthetically by hybridizing two complementary oligonucleotides to. . . a promoter sequence. A single stranded region of at least 5 nucleotides that is complementary to a region of the **nucleic acid** to be sequenced or to a common sequence is introduced by recombinant means at the 3'-end of the coding strand. In preferred embodiments, the promoter-containing **nucleic acid** is covalently coupled via the 3'-end of the noncoding strand or 5'-end of the coding strand to a solid support. . .

SUMM A single stranded 3' overhang of the **nucleic acid** to be sequenced, in single stranded or double stranded form, is hybridized to the complementary sequences of the noncoding strand and, in some embodiments, the nick(s) between one or more **nucleic acid** strands is/are ligated prior to transcription. Transcription is initiated using the appropriate RNA polymerase in the presence of ribonucleoside triphosphates. . .

SUMM When used in array formats, a panel of promoter-containing **nucleic acid** probes may be constructed such that the single stranded complementary regions of the target **nucleic acid** may be permuted along the entire sequence, e.g., the coding sequence of a gene, allowing for the determination of the **nucleic acid** sequence of the entire gene during a single reaction sequence.

SUMM . . . may be identified using mass spectrometric methods. In practicing the methods, a single stranded region of the 3'-end of the **nucleic acid** to be sequenced is hybridized to a complementary sequence at the 3'-end of the coding strand a promoter-containing **nucleic acid** probe. In preferred embodiments, the promoter-containing **nucleic acid** is covalently coupled via the 5'-end of the noncoding strand or 3'-end of the coding strand to a solid support. . .

SUMM . . . 5'-iodo CTP. In certain embodiments, nicks in one or more strand may be ligated by the addition of an appropriate **nucleic acid** ligase prior to initiating transcription (i.e., adding a DNA or RNA ligase). The mass of the terminated RNA transcripts is. . .

DRWD . . . silicon dioxide was reacted with 3-minopropyltriethoxysilane to produce a uniform layer of primary amino groups on the surface. A heterobifunctional **crosslinking agent** was then reacted with the primary amine to incorporate an iodoacetamide-group. An oligodeoxynucleotide containing a 3'- or 5'-disulfide (shown as. . .

DETD As used herein, the term "**nucleic acid**" refers to oligonucleotides or polynucleotides such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) as well as analogs of either RNA or DNA, for

example, made from nucleotide analogs, any of which are in single or double-stranded form. **Nucleic acid** molecules can be synthetic or can be isolated from a particular biological sample using any number of procedures which are. . .

DETD As used herein, a **nucleic acid** promoter-containing probe refers to a **nucleic acid** fragment that includes a double-stranded region encoding a promoter and a single-stranded region that contains at least 5 nucleotides at. . . the coding strand relative to the promoter that is complementary to a single stranded region at the 3'-end of a **nucleic acid** to be sequenced.

DETD As used herein, the target **nucleic acid** is the **nucleic acid** that is sequenced. The target **nucleic acid** will contain or will be modified to contain at least about 5 nucleotides whose sequence is known for hybridization to the immobilized **nucleic acid** promoter-containing probe.

DETD As used herein, **nucleic acid** synthesis refers to any process by which oligonucleotides or polynucleotides are generated, including, but not limited to processes involving chemical. . .

DETD The term "cross-linking agent" is art-recognized, and, as used herein, refers to reagents which can immobilize a **nucleic acid** to an insoluble-support, preferably through covalent bonds. Thus, appropriate "cross-linking agents" for use herein includes a variety of agents that. . . with a functional group present on a surface of the insoluble support and with a functional group present in the **nucleic acid** molecule. Reagents capable of such reactivity include homo- and hetero-bifunctional reagents, many of which are known in the art. Heterobifunctional. . .

DETD As used herein, the terms "protein", "polypeptide" and "peptide" are used interchangeably when referring to a translated **nucleic acid** (e.g. a gene product).

DETD . . . transcription would have occurred if a wild-type or native promoter had been used by the RNA polymerase to transcribe the **nucleic acid** in vitro.

DETD As used herein, a "promoter-containing **nucleic acid**" is a **nucleic acid** that contains a sequence of nucleotides that directs the site-specific binding of an RNA polymerase molecule to form an open. . .

DETD As used herein, a "coding strand" refers to the **nucleic acid** strand of a promoter-containing **nucleic acid** that has the same polarity as a corresponding mRNA molecule initiated from that promoter.

DETD . . . a material used in mass spectrometry that is a proton donating, UV absorbing material, usually organic acid, that forms crystalline matrix-**nucleic acid** structures that are readily ionizable during MALDI. An exemplary matrix material is a solution of 3-hydroxypicolinic acid (3-HPA, 0.7 M. . .

DETD Mass spectrometric methods of sequencing nucleic acids are provided. The sequencing methods use immobilized **nucleic acid** promoter-containing probes that contain a double stranded region encoding a promoter and a single stranded region for hybridizing target nucleic acids. The **nucleic acid** sequence is determined by generating a set of nested base-specific chain terminated RNA transcripts that are analyzed using mass spectrometry.

DETD . . . the art: Qβ replicase (e.g., see U.S. Pat. No. 5,696,249, Re: 35,443 and Eoyang et al. (1971) in Procedures in **Nucleic Acid** Research, Cantoni and Davies, eds., Volume 2, pp. 829-839, Harper and Rowe, N.Y.); bacteria (e.g., E. coli, see Burgess and. . .

DETD The selection of the appropriate RNA polymerase for any given **nucleic acid** template to be sequenced is within the skill of the skilled artisan and varies according to the **nucleic acid** molecule to be sequenced. The selection may be determined empirically following the teachings known to those of skill in the. .

DETD Each **nucleic acid** promoter-containing probe used in the sequencing methods described herein contains a promoter. The promoters used in the methods herein may be obtained from any source, i.e., recombinant or naturally-occurring promoter elements, or may be assembled from synthetic **nucleic acid** oligonucleotide sequences. For example, the **nucleic acid** containing a promoter may be obtained directly from a variety of different organisms, such as bacteria, viruses and eukaryotic organisms,. . . bla or lac promoters, RSV-LTR promoter and F9-1 promoter; Stratagene). The selection of the appropriate promoter will depend on the **nucleic acid** to be sequenced, sequencing conditions, and most importantly, on the RNA polymerase selected for transcription.

DETD Immobilization of **Nucleic Acid** Promoter-containing Probes

DETD In preferred embodiments, the **nucleic acid** promoter-containing probe is immobilized, directly or by means of a cross-linking agent, to a solid support provided herein. Preferred solid. . .

DETD In embodiments of the methods in which a cross-linking reagent is not employed, a modified **nucleic acid** is reacted directly with a appropriately functionalized surface to yield immobilized **nucleic acid**. Thus, for example, an iodoacetyl-modified surface (or other thiol-reactive surface functionality) can react with a thiol-modified **nucleic acid** to provide immobilized nucleic acids.

DETD . . . acids immobilized on the insoluble support. The cross-linking agent (and other reagents used to functionalize the support surface or the **nucleic acid** molecule) can be selected to provide any desired spacing of the immobilized **nucleic acid** molecules from the support surface, and to provide any desired spacing of the immobilized nucleic acids from each other. Thus, steric encumbrance of the **nucleic acid** molecules can be reduced or eliminated by choice of an appropriate cross-linking agent. In certain embodiments, the cross-linking reagent can. . . multiple nucleic acids to a single cross-linking moiety. Preferably, the cross-linking agent is selected to be highly reactive with the **nucleic acid** molecule, to provide rapid, complete, and/or selective reaction. In preferred embodiments, the reaction volume of the reagents (e.g., the thiol. . .

DETD Modified **Nucleic Acid** Promoter-containing Probes and Linkers

DETD Preferred **nucleic acid** promoter-containing probes for use herein are "thiol-modified nucleic acids," i.e., nucleic acids derivatized to contain at least one reactive thiol. . . further detail in Example 1, below, nucleic acids containing at least one reactive thiol are preferably made by treating a **nucleic acid** containing a 3' or 5' disulfide with a reducing agent, which preferably will not compete in subsequent reactions (i.e. will. . . react with an iodoacetimido functionality). Disulfide-derivatized nucleic acids can be synthesized according to a variety of methods. For example, a **nucleic acid** can be modified at the 3'- or 5'-terminus by reaction with a disulfide-containing modifying a reagent. Alternatively, a thiolated primer can by enzymatically or non-enzymatically attached to the **nucleic acid**. A 5'-phosphoramidate functionality can also provide an attachment point for a thiol or disulfide-containing cytosine or deoxycytosine. Examples of reducing agents appropriate for reduction of a disulfide-modified **nucleic acid** include: tris-(2-carboxyethyl)phosphine (TCEP) (preferably a concentration in the range of 1-100 mM (most preferably about 10 mM)) is reacted at. . .

DETD In other embodiments, the **nucleic acid** is immobilized using the photocleavable linker moiety that is cleaved

during mass spectrometry. Exemplary photolabile cross-linker include, but are not. . .

DETD A **nucleic acid** promoter-containing probe can be directly linked to a solid support via a reversible or irreversible bond between an appropriate functionality (L') on the target **nucleic acid** molecule (T) and an appropriate functionality (L) on the capture molecule. A reversible linkage can be such that it is. . .

DETD . . . ammonium group, in which case, preferably, the surface of the solid support carries negative charges which repel the negatively charged **nucleic acid** backbone and thus facilitate the desorption required for analysis by a mass spectrometer. Desorption can occur either by the heat. . .

DETD . . . can serve this purpose and that the donor functionality can be either on the solid support or coupled to the **nucleic acid** molecule to be detected or vice versa.

DETD As noted, at least three version of immobilization are contemplated herein: 1) the target **nucleic acid** is amplified or obtained (the target sequence or surrounding DNA sequence must be known to make primers to amplify or isolated); 2) the primer **nucleic acid** is immobilized to the solid support and the target **nucleic acid** is hybridized thereto to form a promoter sequence; or 3) a double stranded **nucleic acid** encoding a promoter (amplified or isolated) is immobilized through linkage to one predetermined strand, and in vitro transcription is initiated. . .

DETD In the embodiments where the primer **nucleic acid** is immobilized on the solid support and the target **nucleic acid** is hybridized thereto, the inclusion of the cleavable linker allows the primer DNA to be immobilized at the 5'-end so. . .

DETD . . . those of skill in the art for immobilizing nucleic acids to solid supports may be used herein to link the **nucleic acid** to a solid support. The preferred linkers herein are the selectively cleavable linkers, particularly those exemplified herein. Other linkers include,. . .

DETD . . . cross linking reagents that produce photocleavable linkages), thereby releasing the targeted agent upon exposure to light. In preferred embodiments, the **nucleic acid** is immobilized using the photocleavable linker moiety that is cleaved during mass spectrometry.

DETD A variety of chemically cleavable linkers may be used to introduce a cleavable bond between the immobilized **nucleic acid** and the solid support. Acid-labile linkers are presently preferred chemically cleavable linkers for mass spectrometry, especially MALDI-TOF MS, because the acid labile bond is cleaved during conditioning of the **nucleic acid** upon addition of the 3-HPA matrix solution. The acid labile bond can be introduced as a separate linker group, e.g., the acid labile trityl groups or may be incorporated in a synthetic **nucleic acid** linker by introducing one or more silyl internucleoside bridges using diisopropylsilyl, thereby forming diisopropylsilyl-linked oligonucleotide analogs. The diisopropylsilyl bridge replaces. . .

DETD . . . sugar moiety of a nucleotide at positions other than the 3' and 5' position is possible through conventional methods. Also, **nucleic acid** bases can be modified, e.g., as described in F. Eckstein, ed., "Oligonucleotides and Analogues: A Practical Approach," IRL Press (1991).. . .

DETD In preferred embodiments, modification of a **nucleic acid**, e.g., as described above, does not substantially impair the ability of the **nucleic acid** or nucleic sequence to hybridize to its complement. Thus, any modification should preferably avoid substantially modifying the functionalities of the **nucleic acid** which are responsible for Watson-Crick base pairing. The **nucleic acid** can be modified such that a non-terminal thiol group is present, and the **nucleic acid**, when

immobilized to the support, is capable of self-complementary base pairing to form a "hairpin" structure having a duplex region.

DETD . . . rate enzyme turnover. For example, the addition of 4-thio UTP, 5-bromo UTP, 5-iodo CTP alter the hydrogen bonding of the **nucleic acid** facilitating, at least with some RNA polymerases, transcriptional termination and transcript release.

DETD In preferred embodiments, **nucleic acid** promoter-containing probes are immobilized at high densities to the surface of a solid support in an array format. Particularly suitable. . .

DETD In one preferred embodiment, a double stranded **nucleic acid** sequence encoding a promoter sequence is isolated from a natural source e.g., bacteria, viruses, bacteriophages, plants or eukaryotic organisms) or. . . Laboratory Press, New York). This single stranded region is designed such that it is complementary to a region of the **nucleic acid** to be sequenced or to an sequence shared between the two **nucleic acid** molecules (e.g., restriction endonuclease site).

DETD The **nucleic acid** to be sequenced containing at least a partially single stranded 3'-end is hybridized according to the conditions described herein and known to those of skill in the art to the complementary sequences of the promoter-containing DNA. The **nucleic acid** to be sequenced may be single stranded or double stranded. The hybridization of the two **nucleic acid** molecules introduces one or more "nick" in the hybrid at the junction(s) of the adjacent **nucleic acid** molecules. Nicks in the coding or non-coding strand, preferably the coding strand, can be ligated by the addition of an appropriate **nucleic acid** ligase prior to initiating transcription. Methods for ligating nucleic acids are well known to those of skill in the art. . .

DETD . . . method of sequencing may be used for diagnostic applications to determined the presence of genetic alterations in a known target **nucleic acid**. For example, a region of the target **nucleic acid** can be amplified using standard methods, such as PCR or other amplification methods known to those of skill in the. . . art (e.g., see Sambrook et al., (1989) Molecular Cloning, 2nd ed., Cold Spring Harbor Laboratory Press, New York). The amplified **nucleic acid** can be denaturated and the strand to be sequenced, i.e., the noncoding strand, is isolated or may be used as. . .

DETD In preferred embodiments, the **nucleic acid** promoter-containing probe is covalently immobilized on a silica support by functionalization of the support with an amino functionality (e.g., by. . . (Pierce, Rockford, Ill.). Other homo- and hetero-bifunctional reagents which can be employed are available commercially, e.g., from Pierce. Finally, a **nucleic acid** functionalized with a thiol group (e.g., at the 5'-terminus) is covalently bound to the derivatived silica support by reaction of the thiol functionality of the **nucleic acid** molecule with the iodoacetyl functionality of the support.

DETD In certain embodiments, the **nucleic acid** can be reacted with the cross-linking reagent to form a cross-linker/ **nucleic acid** conjugate, which is then reacted with a functionalized support to provide an immobilized **nucleic acid**. Alternatively, the cross-linker can be combined with the **nucleic acid** and a functionalized solid support in one pot to provide substantially simultaneous reaction of the cross-linking reagent with the **nucleic acid** and the solid support. In this embodiment, it will generally be necessary to use a heterobifunctional cross-linker, i.e., a cross-linker with two different reactive functionalities capable of selective reaction with each of the **nucleic acid** and the functionalized solid support.

DETD . . . insoluble support can be selectively cleaved (e.g., by

photolithography) to provide portions of a surface activated for immobilization of a **nucleic acid**. For example, a silicon surface, modified by treatment with 3-mercaptopropyl-triethoxysilane to provide thiol groups, can be blocked with a photocleavable. . . and be selectively deblocked by irradiation of selected areas of the surface, e.g., by use of a photolithography mask. A **nucleic acid** promoter-containing probe modified to contain a thiol-reactive group can then be attached directly to the support, or, alternatively, a thiol-reactive cross-linking reagent can be reacted with the thiol-modified support, followed by (or substantially simultaneously with) reaction with a **nucleic acid** to provide immobilized nucleic acids. A **nucleic acid** base or sequence, once immobilized on a support according to the methods described herein, can be further modified according to known methods. for example, the **nucleic acid** sequence can be lengthened by performing solid-phase **nucleic acid** synthesis according to conventional techniques, including combinatorial technique.

DETD . . . may be identified using mass spectrometric methods. In practicing the methods, a single stranded region of the 3'-end of the **nucleic acid** to be sequenced is hybridized to a complementary sequence at the 3'-end of the coding strand a promoter-containing **nucleic acid** probe. In preferred embodiments, the promoter-containing **nucleic acid** is covalently coupled via the 5'-end of the noncoding strand or 3'-end of the coding strand to a solid support. . .

DETD In certain embodiments, nicks in one or more strand resulting from the hybridization of the **nucleic acid** to be sequenced may be ligated by the addition of an appropriate **nucleic acid** ligase prior to initiating transcription (i.e., adding a DNA or RNA ligase).

DETD . . . hybridized, radiolabeled probes were employed. In cases where a 5'-disulfide-containing oligodeoxynucleotide was to be immobilized, the 3'-terminus was radiolabeled using **terminal transferase** enzyme and a radiolabeled dideoxynucleoside triphosphate; in a standard reaction, 15 pmol (0.6 μM) of the 5'-disulfide-containing oligodeoxynucleotide was incubated. . .

DETD Use of High Density **Nucleic Acid** Immobilization to Generate **Nucleic Acid** Arrays

DETD All primers were synthesized on a commercially available DNA Synthesizer using conventional phosphoroamidite chemistry (Sinha et al. (1984) **Nucleic Acid** Res. 12:4539). In vitro RNA transcription was performed on a synthetic 55 nucleotide double stranded DNA template. The template was. . .

DETD All primers were synthesized on a commercially available DNA Synthesizer using conventional phosphoroamidite chemistry (Sinha et al. (1984) **Nucleic Acid** Res. 12:4539). In vitro RNA transcription was performed on a synthetic 276 nucleotide double stranded DNA template (SEQ ID No.. . .

CLM What is claimed is:
1. A method for determining the sequence of a target **nucleic acid** molecule, comprising: a) immobilizing a **nucleic acid** promoter-containing probe on a solid support, wherein: the **nucleic acid** promoter-containing probe comprises at least 5 nucleotides at the 3'-end of the coding strand that is complementary to a single stranded region at the 3'-end of the target **nucleic acid**, and a double-stranded portion that comprises the promoter, which is oriented to permit transcription of a hybridized target **nucleic acid** molecule; b) hybridizing the target **nucleic acid** to the single-stranded portion of the immobilized **nucleic acid** probe; c) transcribing the target **nucleic acid** with an RNA polymerase to produce a plurality of base-specifically terminated RNA transcripts, wherein the RNA polymerase

recognizes the promoter;. . . the molecular weight value of each base-specifically terminated RNA transcript by mass spectrometry; and e) determining the sequence of the **nucleic acid** by aligning the base-specifically terminated RNA transcripts according to molecular weight.

3. The method of claim 1, wherein the immobilized **nucleic acid** promoter-containing probe is produced by immobilizing a single-stranded molecule that comprises a promoter or the complement of a promoter and. . .

. . . to form a phosphodiester bond between the 3' hydroxyl group and the 5' phosphate group of adjacent strands of the **nucleic acid** probe and the target **nucleic acid**.

10. The method of claim 1, wherein prior to immobilization of the **nucleic acid**, the surface of the support is derivatized by reacting the surface with an aminosilane to produce primary amines on the. . .

14. The method of claim 12, wherein the immobilization of the **nucleic acid** probe to a solid support is effected by reacting the thiol-reactive solid support with a **nucleic acid** probe having a free 5'- or 3'-thiol group, whereby a covalent bond between the thiol group and the thiol-reactive solid. . .

15. The method of claim 1, wherein the **nucleic acid** probe is covalently bound to a surface the solid support at a density of at least 20 fmol/mm.sup.2.

18. The method of claim 1, wherein the surface comprises a plurality of wells comprising the immobilized **nucleic acid** molecule.

25. The method of claim 1, wherein the hybridization of the **nucleic acid** to be sequence to the solid support results in the formation of a nick in the coding strand corresponding to. . .

28. A method of identifying transcriptional terminator sequences or attenuator sequences in a target **nucleic acid** molecule, comprising: a) immobilizing a **nucleic acid** promoter-containing probe on a solid support, wherein the **nucleic acid** promoter-containing probe comprises at least 5 nucleotides at the 3'-end of the coding strand that is complementary to a single stranded region at the 3'-end of the target **nucleic acid**, and a double-stranded portion that comprises the promoter, which is oriented to permit transcription of a hybridized target **nucleic acid** molecule; b) hybridizing the target **nucleic acid** molecule to the immobilized **nucleic acid** probe; c) transcribing the target **nucleic acid** with an RNA polymerase to produce a sequence-terminated RNA transcript, wherein the RNA polymerase recognizes the promoter; and d) determining. . . observed mass of the RNA is indicative of the presence of a the terminator sequence or attenuator in the target **nucleic acid** molecule.

L6 ANSWER 2 OF 2 USPATFULL
AN 1998:157100 USPATFULL
TI Crosslinking oligonucleotides
IN Meyer, Jr., Rich B., Bothell, WA, United States
Gamper, Howard B., Woodinville, WA, United States
Kutyavin, Igor V., Bothell, WA, United States
Gall, Alexander A., Bothell, WA, United States
Petrie, Charles R., Woodinville, WA, United States
Tabone, John C., Bothell, WA, United States

Hurst, Gerald D., The Woodlands, TX, United States
PA Epoch Pharmaceuticals, Inc., Bothell, WA, United States (U.S. corporation)
PI US 5849482 19981215
AI US 1995-485611 19950607 (8)
RLI Continuation-in-part of Ser. No. US 1994-226949, filed on 27 Jun 1994 Ser. No. Ser. No. US 1994-334490, filed on 4 Nov 1994 And Ser. No. US 1994-178733, filed on 7 Jan 1994, now abandoned , said Ser. No. US 226949 which is a continuation-in-part of Ser. No. US 1993-11482, filed on 26 Jan 1993, now abandoned , said Ser. No. US 334490 which is a continuation of Ser. No. US 1993-49807, filed on 20 Apr 1993, now abandoned which is a continuation of Ser. No. US 1989-353857, filed on 18 May 1989, now abandoned which is a continuation-in-part of Ser. No. US 1988-250474, filed on 28 Sep 1988, now abandoned , said Ser. No. US 178733 which is a continuation of Ser. No. US 1991-748138, filed on 21 Aug 1991, now abandoned which is a continuation-in-part of Ser. No. US 353857 which is a continuation-in-part of Ser. No. US 250474
DT Utility
FS Granted
LN.CNT 2190
INCL INCLM: 435/006.000
INCLS: 536/023.100; 536/024.300
NCL NCLM: 435/006.000
NCLS: 536/023.100; 536/024.300
IC [6]
ICM: C12Q001-68
ICS: C07H021-04
EXF 514/44; 536/24.3; 536/24.5; 536/24.31; 536/24.32; 536/18.4; 536/124; 536/23.1; 435/6; 435/172.1; 435/172.3; 435/375; 436/94; 436/183
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

SUMM . . . thus interfere with the replication or transcription of selected target genes. As is known, except for certain RNA viruses and **nucleic acid**-free viroids, DNA is the repository for all genetic information, including regulatory control sequences and non-expressed genes, such as dormant proviral. . .

SUMM . . . to inhibit restriction and/or transcription of the target double stranded DNA. Based on the known stabilities of the two target **nucleic acid** species (i.e., DNA and RNA), anti-gene interference with DNA functioning has longer lasting effects than the corresponding antisense inhibition of. . .

SUMM . . . chain of nucleotides which are linked to one another by phosphate ester linkages. Each nucleotide typically comprises a heterocyclic base (**nucleic acid** base), a sugar moiety attached to the heterocyclic base, and a phosphate moiety which esterifies a hydroxyl function of the. . .

SUMM . . . sugars or sugar analogs and of the respective "nucleosides" wherein such sugars or analogs are attached to a heterocyclic base (**nucleic acid** base) per se is known, and need not be described here, except to the extent such preparation is provided here. . .

SUMM The heterocyclic bases, or **nucleic acid** bases which are incorporated in the modified ODNs of the present invention may be the naturally occurring principal purine and. . .

SUMM Other examples of nucleotides where the **crosslinking agent** is attached to a heterocyclic base, are 2'-deoxy-4-aminopyrazolo[3,4-d]pyrimidine derivatives. The general structure of these derivatives is shown below in Formula. . .

SUMM . . . include ligands which bind to antibodies labeled with fluorophores, chemiluminescent agents, and enzymes. Alternatively, probes can be conjugated directly with **labels** such as fluorophores, chemiluminescent agents, enzymes and enzyme substrates. Alternatively, the same components may be indirectly bonded through a ligand-antiligand. . .

SUMM . . . for example, by using DNA synthesizers, by nick-translation, by

tailing of radioactive bases in the 3' end of probes with **terminal transferase**, by copying M13 plasmids having specific inserts with the **Klenow fragment** of **DNA polymerase** in the presence of radioactive dNTP's, or by transcribing RNA from templates using RNA polymerase in the presence of radioactive. . .

SUMM The particular hybridization technique is not essential to the invention. Hybridization techniques are generally described in " **Nucleic Acid** Hybridization, A Practical Approach", Hames and Higgins, Eds., IRL Press, 1985; Gall and Pardue, Proc. Natl. Acad. Sci., U.S.A., 63:378-383. . .

SUMM . . . present in the hybridization solution may vary widely. Generally, substantial excess of probe over the stoichiometric amount of the target **nucleic acid** will be employed to enhance the rate of binding of the probe to the target DNA or RNA.

SUMM This first aspect of the invention is also directed to a method or identifying target single stranded **nucleic acid** sequences, which method comprises utilizing an oligonucleotide probe including at least one ODN having a cross-linking agent and a label. . .

SUMM . . . one labeled ODN having a cross-linker covalently attached, wherein the ODN comprises a sequence complementary to that of the target **nucleic acid** sequence;

SUMM An assay for identifying target single stranded **nucleic acid** sequences utilizing a labeled oligonucleotide probe including the covalently attached cross-linking agent and comprising the above method is contemplated for. . . probe reagent (ODN) having a sequence complementary to that of the target nucleic acids; a denaturation reagent for converting double-stranded **nucleic acid** to a single-stranded **nucleic acid**; and a hybridization reaction mixture. The kit can also include a signal-generating system, such as an enzyme for example, and. . .

DETD **DNA polymerase** 1 (U.S. Biochemicals)--8 U/mL

DETD . . . mL), nucleotide mix A (6 mL), Bio-12-dAPPTP (2 mL), and H.sub.2 O (20 mL) was added DNase (1 mL) and **DNA polymerase** 1 (2.4 mL). The reaction mixture was incubated at 16° C. for 1 hour. The procedure was repeated using Bio-11dUTP. . .

DETD **Nucleic acid** was isolated by ethanol precipitation and hybridized to pHPV-16 slotted onto nitrocellulose. The hybridized biotinylated probe was visualized by a. . .

DETD The reaction of crosslinking a DNA probe to a target **nucleic acid** sequence contained 1 μg of haloacylamidoalkyl probe and 10 ng of .sup.32 P-labeled cordycepin-tailed target in 200 μL of 0.1M. . .

DETD . . . with the HPV system of Example 25 where U is 5-(3-iodoacetamidoprop-1-yl)-2'-deoxyuridine. The target was .sup.32 P-labeled by cordycepin tailing with **terminal transferase** (Maniatis et al., "Molecular Cloning--A Laboratory Manual", Cold Spring Harbor Laboratory, 1982, p. 239) and incubated with excess probe in. . .

DETD . . . the sugar or any heterocyclic base within the ODN. A cross-linking agent which has two cross-linking functionalities, such as a **crosslinking agent** having the formula --N--[(CH.sub.2).sub.2 --L].sub.2 (a bifunctional N-mustard) is capable of two alkylations, and is therefore considered as two cross-linking. . .

DETD (2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
CTGGATGTUC CTTC 14

DETD (2) INFORMATION FOR SEQ ID NO:2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
AGACAGCACA GAATTCGAAG GAACATCCAG 30
DETD (2) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
ACCGTCCTTG ACACGATGGA CTCC 24
DETD (2) INFORMATION FOR SEQ ID NO:4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
CTCCAUCGTG TCAAG 15
DETD (2) INFORMATION FOR SEQ ID NO:5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
NAGAGGAGAA AGGAGAGAGN 20
DETD (2) INFORMATION FOR SEQ ID NO:6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
ATATAAGGAG AGAGGAAAGA GGAGACAAA 29
DETD (2) INFORMATION FOR SEQ ID NO:7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
TTGTGGTGGT YGTGTYGTGG TGGG 24
DETD (2) INFORMATION FOR SEQ ID NO:8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
GGGAGGAGCA GAGGAGGAGG AGAA 24
DETD (2) INFORMATION FOR SEQ ID NO:9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
TTTTCTTTTY GGGGGTN 17

DETD (2) INFORMATION FOR SEQ ID NO:10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
TTTTTAAAAG AAAAGGGGGG ACTGG 25
DETD (2) INFORMATION FOR SEQ ID NO:11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
NCTTTCCTCT CTTTTCCCCN 20
DETD (2) INFORMATION FOR SEQ ID NO:12:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
AAATACTGGG AGAAAGGAGA GAAAAGGGGA CCCAACGTAT 40
DETD (2) INFORMATION FOR SEQ ID NO:13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 272 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
CGGGAAGCTA GAGTAAGTAG TTCGCCAGTT AATAGTTTGC GCAACGTTGT TGCCATTGCT 60
ACAGGCATCG TGGTGTCACG CTCGTCGTTT GGTATGGCTT CATTCAGCTC CGGTTCCCAA. . .
DETD (2) INFORMATION FOR SEQ ID NO:14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
ATGTTGTGCA AAAAAGCGGT TAGCTCCTTC GGTCCUCCGA TCGTTGTCAG 50
DETD (2) INFORMATION FOR SEQ ID NO:15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:
ATGTTGTGCA AAAAAGCGGT TAGCTCCTTC GGTCCTCCGA TCGTTGTCAG 50
DETD (2) INFORMATION FOR SEQ ID NO:16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:
AAAAAGCGGT TAGCTCCTTC GGTCCUCCGA TCGTTGTCAG 40
DETD (2) INFORMATION FOR SEQ ID NO:17:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TAGCTCCTTC GGTCCUCCGA TCGTTGTCAG 30
DETD (2) INFORMATION FOR SEQ ID NO:18:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:
GGTCCUCCGA TCGTTGTCAG 20
DETD (2) INFORMATION FOR SEQ ID NO:19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:
CCACCACATC GCCGCATAAC CGATCCCTTC GGTCCUCCGA TCGTTGTCAG 50
DETD (2) INFORMATION FOR SEQ ID NO:20:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:
ATGTTGTGCA AAAAAGCGGT TAGCTTTCCT AACTTUTTAC CTACCACTGA 50
DETD (2) INFORMATION FOR SEQ ID NO:21:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:
CCACCACATC GCCGCATAAC TAGCTCCTTC GGTCCUCCGA TCGTTGTCAG 50
DETD (2) INFORMATION FOR SEQ ID NO:22:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:
ATGTTGTGCA AAAAAGCGGT TAGCTCCTTC AACTTUTTAC CTACCACTGA 50
DETD (2) INFORMATION FOR SEQ ID NO:23:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:
AAAAGCGGTT AGCTCCTTCG GTCCUCCGAT CGTTGTCAGA AGTAAGTTG 49
DETD (2) INFORMATION FOR SEQ ID NO:24:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:
AAAAGCGGTT AGCTCCTTCG ACCCUCCACT CGTTGTCAGA AGTAAGTTG 49
DETD (2) INFORMATION FOR SEQ ID NO:25:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

AAAAGCGGTT AGCTCCTTCG ACTCUCTACT CGTTGTCAGA AGTAAGTTG 49
DETD (2) INFORMATION FOR SEQ ID NO:26:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:
AAAAGCGGTT AGCTCCTTCG ACTTUTTACT CGTTGTCAGA AGTAAGTTG 49
DETD (2) INFORMATION FOR SEQ ID NO:27:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:
GGTTATTTTT GAAGATACGA ATTTCUCCAG AGACACAGCA GGATTTGTCA 50
DETD (2) INFORMATION FOR SEQ ID NO:28:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:
GAAGATACGA ATTTCUCCAG AGACACAGCA 30
DETD (2) INFORMATION FOR SEQ ID NO:29:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: **nucleic acid**
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:
CCCCCATGTT GTGCAAAAAA GCGGTTAGCT CCTTCGGTCC TCCGATCGTT GTCAGAAGTA 60
AGTTGGCCGC A 71

```
L7   ANSWER 1 OF 3  CAPLUS  COPYRIGHT 2002 ACS
AN   2000:608932  CAPLUS
DN   133:190215
TI   Methods for making morpholino-nucleotides, and their use for analyzing and
     marking nucleic acid sequences
IN   Marciacq, Florence; Sauvaigo, Sylvie; Mouret, Jean-Francois; Issartel,
     Jean-Paul; Molko, Didier
PA   Commissariat A L'Energie Atomique, Fr.; Centre National De La Recherche
     Scientifique
SO   PCT Int. Appl., 73 pp.
     CODEN: PIXXD2
DT   Patent
LA   French
IC   ICM  C12P019-34
     ICS  C12Q001-68; C07H021-00
FAN.CNT 1
     PATENT NO.       KIND  DATE           APPLICATION NO.  DATE
     ---------------  ----  --------       ---------------  --------
PI   WO 2000050626     A1   20000831       WO 2000-FR427    20000221
         W:  CA, JP, US
         RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,
             PT, SE
     FR 2790004        A1   20000825       FR 1999-2170     19990222
     FR 2790005        A1   20000825       FR 1999-12001    19990927
     EP 1155140        A1   20011121       EP 2000-906441   20000221
         R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
             IE, FI
PRAI FR 1999-2170      A    19990222
     FR 1999-12001     A    19990927
     WO 2000-FR427     W    20000221
OS   CASREACT 133:190215; MARPAT 133:190215
RE.CNT  5       THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
                ALL CITATIONS AVAILABLE IN THE RE FORMAT
ST   morpholino nucleotide analog DNA sequence analysis

L7   ANSWER 2 OF 3  CAPLUS  COPYRIGHT 2002 ACS
AN   1966:466676  CAPLUS
DN   65:66676
OREF 65:12453e-f
TI   Isonicotinyl hydrazones of nucleosides and their phosphorylated
     derivatives
AU   Midgley, J. E. M.
CS   Univ. Leeds, UK
SO   Biochim. Biophys. Acta (1966), 123(1), 210-13
DT   Journal
LA   English
IT   3'-Cytidylic acid, 6-ester with N-[2-(2-amino-1,6-dibydro-6-oxo-9H-purin-9-
        yl)-3,5-dihydroxy-6-(hydroxymethyl)morpholino]isonicotinamide
     Isonicotinamide, N-[2-(4-amino-2-oxo-1(2H)-pyrimidinyl)-3,5-dihydroxy-6-
        (hydroxymethyl)morpholino]-, nucleotide esters
     Isonicotinamide, N-[2-(6-amino-2-oxo-1(2H)-pyrimidinyl)-3,5-dihydroxy-6-
        (hydroxymethyl)morpholino]-, 6-esters with 3'-guanylic and 3'-uridylic
        acids
        (hydrolysis by ribonuclease)

L7   ANSWER 3 OF 3  USPATFULL
AN     2002:69973  USPATFULL
TI     p53 antisense agent and method
IN     Iversen, Patrick L., Corvallis, OR, United States
PA     AVI BioPharma, Inc., Corvallis, OR, United States (U.S. corporation)
PI     US 6365577          B1   20020402
AI     US 1999-426804           19991022 (9)
PRAI   US 1998-105695P      19981026 (60)
DT     Utility
```

FS GRANTED
LN.CNT 1006
INCL INCLM: 514/044.000
INCLS: 435/006.000; 435/091.100; 435/375.000; 435/455.000; 536/023.100; 536/024.500; 536/025.300; 536/031.000
NCL NCLM: 514/044.000
NCLS: 435/006.000; 435/091.100; 435/375.000; 435/455.000; 536/023.100; 536/024.500; 536/025.300; 536/031.000
IC [7]
ICM: A01N043-04
ICS: C12Q001-68; C12N015-63; C07H021-00; C08B003-00
EXF 435/6; 435/91.1; 435/455; 435/366; 435/375; 536/23.1; 536/24.5; 536/31; 536/25.3; 514/44
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
DETD . . . . A 1 μmol silica gel support column with the 3' base of the ODN (i.e. unmodified nucleotide, C-5-propyne nucleotide, or **morpholino nucleotide** analog) linked by the 3' hydroxy group is inserted, and synthesis is carried out in a base by base fashion. . .

```
L6   ANSWER 1 OF 2  USPATFULL
AN      2001:121238  USPATFULL
TI      Mass spectrometric methods for sequencing nucleic acids
IN      Kang, Changwon, Taejon, Korea, Republic of
        Kwon, Young-Soo, Kwangju, Korea, Republic of
        Kim, Young Tae, Seoul, Korea, Republic of
        Koster, Hubert, La Jolla, CA, United States
        Little, Daniel P., Patton, PA, United States
        Little, Maryanne J., Groton, MA, United States  now by change of name
        from Maryanne J. O'Donnell
        Xiang, Guobing, San Diego, CA, United States
        Lough, David M., Eyemouth, United Kingdom
        Cantor, Charles, Boston, MA, United States
PA      Sequenom, Inc., San Diego, CA, United States (U.S. corporation)
PI      US 6268131          B1    20010731
AI      US 1997-990851            19971215 (8)
DT      Utility
FS      GRANTED
LN.CNT 3013
INCL    INCLM: 435/006.000
        INCLS: 435/091.200
NCL     NCLM:  435/006.000
        NCLS:  435/091.200
IC      [7]
        ICM: C12Q001-68
EXF     435/6; 435/91.1; 435/91.2; 435/91.3; 435/518; 435/528; 436/518; 436/528;
        536/23.1
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6   ANSWER 2 OF 2  USPATFULL
AN      1998:157100  USPATFULL
TI      Crosslinking oligonucleotides
IN      Meyer, Jr., Rich B., Bothell, WA, United States
        Gamper, Howard B., Woodinville, WA, United States
        Kutyavin, Igor V., Bothell, WA, United States
        Gall, Alexander A., Bothell, WA, United States
        Petrie, Charles R., Woodinville, WA, United States
        Tabone, John C., Bothell, WA, United States
        Hurst, Gerald D., The Woodlands, TX, United States
PA      Epoch Pharmaceuticals, Inc., Bothell, WA, United States (U.S.
        corporation)
PI      US 5849482                19981215
AI      US 1995-485611            19950607 (8)
RLI     Continuation-in-part of Ser. No. US 1994-226949, filed on 27 Jun 1994
        Ser. No. Ser. No. US 1994-334490, filed on 4 Nov 1994 And Ser. No. US
        1994-178733, filed on 7 Jan 1994, now abandoned , said Ser. No. US
        226949 which is a continuation-in-part of Ser. No. US 1993-11482, filed
        on 26 Jan 1993, now abandoned , said Ser. No. US 334490 which is a
        continuation of Ser. No. US 1993-49807, filed on 20 Apr 1993, now
        abandoned which is a continuation of Ser. No. US 1989-353857, filed on
        18 May 1989, now abandoned which is a continuation-in-part of Ser. No.
        US 1988-250474, filed on 28 Sep 1988, now abandoned , said Ser. No. US
        178733 which is a continuation of Ser. No. US 1991-748138, filed on 21
        Aug 1991, now abandoned which is a continuation-in-part of Ser. No. US
        353857 which is a continuation-in-part of Ser. No. US 250474
DT      Utility
FS      Granted
LN.CNT 2190
INCL    INCLM: 435/006.000
        INCLS: 536/023.100; 536/024.300
NCL     NCLM:  435/006.000
        NCLS:  536/023.100; 536/024.300
IC      [6]
        ICM: C12Q001-68
```